201-14218

To: oppt.ncic@epamail.epa.gov, Rtk Chem/DC/USEPA/US@EPA
cc: Stephen Dimond <stephen.dimond@gep.ge.com>, Fisher Louan <lfisher@toxregserv.com>, "Ronald L Joiner (GEP)" <Ronald.Joiner@gepex.ge.com>

Subject: Submission of Test Plans/Robust Summaries for General Electric Company - Plastics (GE Plastics; Ref # 1100342)

Please find attached, a Transmittal Letter and Test Plans/Robust Summaries for the following:

4-Nitro-N-Methylphthalimide (CAS RN 41663-84-7)
2,4,6-Trimethylphenol (CAS RN 527-60-6)
N-Methylphthalimide (CAS RN 550-44-7)

These submissions are made on behalf of General Electric Company - Plastics (GE Plastics;). The files are in PDF format.

A hard copy of these Test Plans/Robust Summaries and electronic copies on a disk were sent to the attention of the Chemical Right-to Know Program yesterday, December 30, 2002 from Toxicology/Regulatory Services (TRS). We have subsequently determined that this method of electronic submission is more effective and ask that you disregard the files sent via disk upon their receipt.

Your help is appreciated. Thank you.

John P. Van Miller, Ph.D., DABT
Toxicology/Regulatory Services
2365 Hunters Way
Charlottesville, VA 22911
Phone: 434-977-5957
Fax: 434-977-0899
EMAIL: jvanmiller@toxregserv.com

2,4,6-Trimethylphenol _527-60-6_-Test Plan.pdf 4-Nitro-N-Methylphthalimide _41663-84-7_-Test Plan.pdf
GE HPV Transmittal Letter-123002.pdf N-Methyl-Phthalimide _550-44-7_-Test Plan.pdf

TRS
TOXICOLOGY/REGULATORY SERVICES, INC.

December 30, 2002

Christine Todd Whitman, Administrator
US Environmental Protection Agency
PO Box 1473
Merrifield, VA 22116

Attention: Chemical Right-to-Know Program, AR-201

Re: Test Plans for CAS RNs 41663-84-7, 527-60-6 and 550-44-7

Toxicology/Regulatory Services (TRS) is submitting three Test Plans/Robust Summaries on behalf of General Electric Company – Plastics (GE Plastics; Registration Number). Please add the attached Test Plans and Robust Summaries for 4-Nitro-N-Methylphthalimide (CAS RN 41663-84-7), 2,4,6-Trimethylphenol (CAS RN 527-60-6) and N-Methylphthalimide (CAS RN 550-44-7) sponsored by GE Plastics to the list of those chemicals to begin testing in 2002. The Test Plan/Robust Summaries are in Microsoft Word 2000 format. Please address any comments to:

Dr. Ronald Joiner
Manager, Global Toxicology
General Electric Company
One Plastics Avenue
Pittsfield, MA 01201
Phone: 413-448-6323; Fax: 413-448-6590
EMAIL: Ronald.Joiner@GEP.GE.COM

Thank you,

John P. Van Miller

(Digitally signed by John P. Van Miller
DN: cn=John P. Van Miller, c=US
Date: 2002.12.30 15:48:41 -05'00')

John P. Van Miller, Ph.D., DABT

Enclosures

Cc: Dr. Ronald L. Joiner, GE Plastics (without enclosures)
Mr. Stephen S. Dimond, GE Plastics (without enclosures)

2365 HUNTERS WAY, CHARLOTTESVILLE, VIRGINIA 22911 ■ TELEPHONE: 434-977-5957 ■ FACSIMILE: 434-977-0899
EMAIL: trs@toxregserv.com ■ WEBSITE: www.toxregserv.com

201-14218A

U.S. HIGH PRODUCTION VOLUME (HPV)
CHEMICAL CHALLENGE PROGRAM

ROBUST SUMMARY

2,4,6-Trimethylphenol (CAS RN 527-60-6)

Prepared by:
General Electric Company
Pittsfield, MA, USA

Prepared for:
U.S. Environmental Protection Agency
Washington, D.C., USA

December 30, 2002

Table of Contents

2,4,6-Trimethylphenol [CAS RN 527-60-6] Page

CHEMICAL IDENTITY AND USE INFORMATION

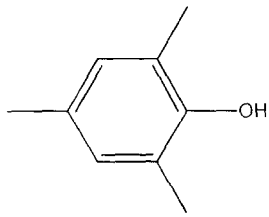
CAS RN:

527-60-6

CHEMICAL NAME:

2,4,6-Trimethylphenol

STRUCTURE, MOLECULAR WEIGHT, FORMULA:



Molecular Formula: $C_9H_{12}O$
Molecular Wt.: 136.19

OTHER CHEMICAL IDENTITY INFORMATION

1-Hydroxy-2,4,6-trimethylbenzene
2-Hydroxymesitylene
Benzene, 2-hydroxy-1,3,5-trimethyl-
Mesitol
Mesityl alcohol
Phenol, 2,4,6-trimethyl-

QUANTITY PRODUCED PER YEAR

Approximately 13 million pounds per year with 75% burned on the manufacturing site.

USE PATTERN

2,4,6,-Trimethylphenol is used by one manufacturer of insulating varnishes for the magnet wire industry. These varnishes are included in a resin to be deposited on the wire suspended in the solvent. Formulations of 33% m/p-cresol, 33% mesitol, and 34% phenol are typically used in the industry as solvents for either polyimide or polyurethane based resin to coat wire. The coating provides ease of use, insulation, and durability to the wire surface. Of the 13 MM lbs produced in 2001, about 3 MM lbs was sold to two customers, with the remaining amount burned onsite.

TEST PLAN

2,4,6-Trimethylphenol CAS RN: 527-60-6		Information	OECD Study	GLP	Other Study	Estimation Method	Acceptable	Testing Required
STUDY		**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**
PHYSICAL AND CHEMICAL DATA								
1.0	Melting Point	Y	N	N	Y	N	Y	N*
2.0	Boiling Point	Y	N	N	Y	N	Y	N*
3.0	Vapour Pressure	Y	N	N	Y	N	Y	N*
4.0	Partition Coefficient	Y	N	N	Y	N	Y	N*
5.0	Water Solubility	Y	N	N	Y	N	Y	N*
ENVIRONMENTAL FATE AND PATHWAY								
6.0	Photodegradation	Y	N	N	Y	Y	Y	N
7.0	Stability in Water	Y	Y	N	N	Y	Y	N
8.0	Transport and Distribution	Y	N	N	Y	Y	Y	N
9.0	Biodegradation	Y	N	N	Y	N	N	Y
ECOTOXICITY								
10.0	Acute Toxicity to Fish	Y	Y	Y	N	N	Y	N
11.0	Toxicity to Algae	Y	Y	Y	N	N	Y	N
12.0	Acute Toxicity to Daphnia	Y	Y	Y	N	N	Y	N
TOXICITY								
13.0	Acute Toxicity	Y	Y	Y	N	N	Y	N
14.0	Genotoxicity *In Vitro or In Vivo* (Chromosome Aberration Tests)	Y	Y	Y	N	N	Y	N
15.1	Genotoxicity *In Vitro* (Bacterial Test)	Y	N	N	Y	N	Y	N
15.2	Genotoxicity *In Vitro* (Mammalian Cells)	Y	Y	Y	N	N	Y	N
16.0	Repeated Dose Toxicity	N	N	N	N	N	N	Y**
17.0	Reproductive Toxicity	N	N	N	N	N	N	Y**
18.0	Development Toxicity / Teratogenicity	N	N	N	N	N	N	Y**

* These studies are acceptable for the High Production Volume Chemicals Challenge Program but are being repeated to ensure accuracy and completeness.

** OECD 422 study to be conducted to fulfill these endpoints.

ROBUST SUMMARY

PHYSICAL AND CHEMICAL DATA

1.0 MELTING POINT

Value:	73 °C
Decomposition:	Yes [ ] No [ ] Ambiguous [X]
Sublimation:	Yes [ ] No [ ] Ambiguous [X]
Method:	Not specified
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	Melting point value found in the published literature via the National Library of Medicine's Hazardous Substance Databank.
Reference:	Lide, D.R. (1996) CRC Handbook of Chemistry and Physics. 76th Edition, Boca Raton, FL. CRC Press Inc. pp: 3-259.
Reliability:	(Klimisch Code 2) Reliable with restrictions. Test method, GLP status, and test article source and purity unknown.

2.0 BOILING POINT

Value:	220°C
Decomposition	Yes [ ] No [ ] Ambiguous [X]
Method:	Not specified
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	Boiling point value found in the published literature via the National Library of Medicine's Hazardous Substance Databank.
Reference:	Lide, D.R. (1996) CRC Handbook of Chemistry and Physics. 76th Edition, Boca Raton, FL. CRC Press Inc. pp: 3-259.
Reliability:	(Klimisch Code 2) Reliable with restrictions. Test method, GLP status, and test article source and purity unknown.

3.0 VAPOR PRESSURE

Value:	0.05 mm Hg
Temperature:	25°C
Decomposition:	Yes [ ] No [ ] Ambiguous [X]
Method (Year):	Calculated [ ]; Measured [ ]; Unknown [X]
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	Vapor pressure found in the published literature via the National Library of Medicine's Hazardous Substance Databank.
Reference:	Boublik, T., Fried, V., and Hala, E. (1984). The vapour pressures of pure substances. 2nd Revised Edition. Amsterdam, Elsevier, pp. 729.
Reliability:	(Klimisch Code 2) Reliable with restrictions. Test method, GLP status, and test article source and purity unknown.

4.0 PARTITION COEFFICIENT ($Log_{10}P_{ow}$)

4.0.1

Log Kow:	2.73
Temperature:	Not specified
Method (Year):	Calculated [ ]; Measured [ ]; Unknown [X]
GLP:	Yes [ ] No [ ] ? [X]

Remarks:	Octanol/water partition coefficient value found in the published literature via the National Library of Medicine's Hazardous Substance Databank.
Reference:	Sangster, J. (1989). *J. Phys. Chem. Ref. Data*. 18:1111-1230.
Reliability:	(Klimisch Code 2) Reliable with restrictions. Test method, temperature, GLP status, and test article source and purity unknown not specified.

5.0 WATER SOLUBILITY

5.1 SOLUBILITY

Value:	1.01 x 10^3 mg/L
Temperature:	25 °C
Description:	Miscible [ ]; Of very high solubility [ ]; Of high solubility [ ]; Soluble [X]; Slightly soluble [ ]; Of low solubility [ ]; Of very low solubility [ ]; Not soluble [ ]
Method (Year):	Not specified
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	Water solubility value found in the published literature via the National Library of Medicine's Hazardous Substance Databank.
Reference:	Shiu et al. (1994) *Chemosphere*, 29:1155-1224.
Reliability:	(Klimisch Code 2) Reliable with restrictions. Test method, water pH, GLP status, and test article source and purity unknown.

5.2. pH VALUE, pKa VALUE

No studies were found.

ENVIRONMENTAL FATE AND PATHWAYS

6.0 PHOTODEGRADATION

Synopsis of a report entitled* "Fugacity Modeling to Estimate Transport Between Environmental Compartments for 2,4,6-Trimethylphenol (TMP) (CAS Reg. No. 527-60-6)*, dated December 17, 2002 for General Electric Company, Pittsfield, MA, USA from Charles A. Staples, Ph.D., Assessment Technologies, Inc. Fairfax, VA, USA.

Reported here are the results of fugacity-based distribution modeling conducted for 2,4,6-trimethylphenol (TMP). Physical properties data were taken from robust summaries prepared for melting and boiling points, vapor pressure, octanol-water partitioning, and aqueous solubility. Henry's Law constant was calculated from vapor pressure, water solubility and molecular weight. This document contains a brief synopsis of physical property and environmental fate property estimation, fugacity-based distribution modeling, and modeling results for TMP.

Fugacity-based Distribution Modeling

Introduction

Fugacity-based distribution modeling requires several physical properties and fate characteristics as model inputs. Property estimation programs were used to obtain estimates of any physical property or fate characteristic (e.g., atmospheric photo-oxidation and

biodegradation) for which data were not provided. To estimate the physical properties and fate characteristics, several models can be employed. The models were based on structure-activity relationships (SAR) and were used to estimate hydroxyl radical mediated atmospheric photo-oxidation for TMP.

Methods for Estimating Physical Properties

The SAR models for estimating physical properties and abiotic degradation were developed by the U.S. Environmental Protection Agency and Syracuse Research Corporation (Estimation Programs Interface for Windows, Version 3.05 or EPIWIN v.3.05) (Syracuse 2000). The models can be used to calculate melting point, vapor pressure (submodel MPBPVP), octanol-water partition coefficient or Kow (submodel KOWWIN), and aqueous solubility (submodel WSKOWWIN). The calculation procedures are described in the program guidance and are adapted from standard procedures based on analysis of key structural features (Meylan and Howard, 1999a,b,c). Key assumptions and default parameters used in the models were developed under U.S. EPA guidance. EPA uses the models for various regulatory activities.

Methods for Estimating Environmental Fate

Atmospheric photo-oxidation potential was estimated using the submodel AOPWIN (Meylan and Howard, 2000). The estimation methods employed by AOPWIN are based on the SAR methods developed by Dr. Roger Atkinson and co-workers (Meylan and Howard, 2000a). The SAR methods rely on structural features of the subject chemical. The model calculates a second-order half-life with units of cm^3/molecules-sec. Photo-degradation based on atmospheric photo-oxidation is in turn based on the second order rate of reaction (cm^3/molecules-sec) with hydroxyl radicals (HO•), assuming first-order kinetics and an HO• concentration of 1.5 E+6 molecules/cm^3 and 12 hours of daylight. Pseudo-first order half-lives ($t_{1/2}$) were then calculated as follows: $t_{1/2} = 0.693 / [k_{phot} \times HO\bullet \times 12\text{-hr} / 24\text{-hr}]$.

Biodegradation potential is estimated by EPIWIN using BIOWIN v4.00 (Meylan and Howard, 2000b). BIOWIN estimates the biodegradation potential of a compound using six methods (Linear and Non-Linear models, Ultimate and Primary Surveys, and MITI Linear and Non-Linear models). All models rely on structure-activity to provide numerical estimates. From the numerical estimates, a qualitative expression of biodegradation potential is made (e.g., "fast").

Estimation of Environmental Distributions

The fugacity-based distribution model was based on the Trent University Modeling Center's EQuilibrium Concentration model (EQC) Level 3 model, version 1.01. These models are described in Mackay et al. (1996a,b). Fugacity-based modeling is based on the “escaping” tendencies of chemicals from one phase to another. For instance, a Henry’s Law constant calculated from aqueous solubility and vapor pressure is used to describe the “escape” of a chemical from water to air or vice versa as it seeks to attain equilibrium between the phases. The key physical properties required as input parameters into the model are melting point, vapor pressure, octanol-water partition coefficient (Kow), and aqueous solubility. The model also requires estimates of first-order half-lives in air, water, soil, and sediment. An additional key input parameter is loading or emissions of the chemical into the environment. The default assumption was used here, which assumes equal releases to air, water, and soil. The model was run using the chemical specific parameters to obtain estimates of the chemical distributions between environmental compartments.

Common Features of the Models

All of the models use the structure of the molecule to begin performing the calculation. The structure must be entered into the model in the form of a SMILES notation or string

(Simplified Molecular Input Line Entry System). It is a chemical notation system used to represent a molecular structure by a linear string of symbols. The SMILES string allows the program to identify the presence or absence of various structural features that control aspects of the submodels. The models do contain structures and SMILES strings for about 100,000 compounds, accessible via Chemical Abstracts Service (CAS) Registry number.

Model Results for TMP

All physical properties data were taken directly from the robust summaries. For TMP, the following data have been reported and were used to conduct fugacity-based distribution modeling. TMP has a melting point averaging 73.0°C, a vapor pressure of 0.050 mm Hg (6.66 Pa) at 25°C, a log Kow value of 2.73, and a water solubility of 1010 mg/L (25°C). Additional parameters were estimated using EPIWIN models. For abiotic processes, TMP does not undergo hydrolysis but is subject to photooxidation in the atmosphere. They include an atmospheric half-life of 10.5 hours, water and soil biodegradation half-lives of 900 hours, and a sediment half-life of 3600 hours. These values were all used in the distribution modeling. The results of the distribution modeling (assuming equal emissions to air, water, and soil) were: Air 0.773%, Water 31.7%, Soil 67.2%, and Sediment 0.336%.

References

Mackay, D. et al. 1996a. Assessing the fate of new and existing chemicals: a five-stage process. *Environ. Toxicol. Chem*. 15(9): 1618-1626.

Mackay,D. et al. 1996b. Evaluating the environmental fate of a variety of types of chemicals using the EQC model. *Environ. Toxicol. Chem*. 15(9): 1627-1637.

Meylan, W. and PH Howard. 1999a. User's Guide for MPBPVP, Version 1.4. Syracuse Research Corporation. North Syracuse, New York. December, 1999.

Meylan, W. and PH Howard. 1999b. User's Guide for KOWWIN, Version 1.6. Syracuse Research Corporation. North Syracuse, New York. July, 1999.

Meylan, W. and PH Howard. 1999c. User's Guide for WSKOWWIN, Version 1.3. Syracuse Research Corporation. North Syracuse, New York. April, 1999.

Meylan, W. and PH Howard. 2000a. User's Guide for AOPWIN, Version 1.9. Syracuse Research Corporation. North Syracuse, New York. March, 2000.

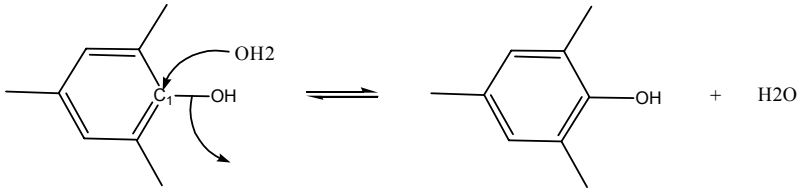
Meylan, W. and PH Howard. 2000b. User's Guide for BIOWIN, Version 4.0. Syracuse Research Corporation. North Syracuse, New York. February, 2000.

Syracuse Research Corporation. 2000. User's Guide for Estimation Programs Interface for Windows, Version 3, Syracuse Research Corporation. North Syracuse, New York.

7.0 STABILITY IN WATER

Type:	Abiotic (hydrolysis) [ ]; biotic (sediment) [ ]; N/A [X]
Half life:	N/A (see remarks below)
Degradation:	N/A (see remarks below)
Method:	OECD Test Guideline 111 (1993)
GLP:	Yes [ ] No [X] ? [ ]
Test Substance:	2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6)
Remarks:	Of the compounds containing carbon, hydrogen, and oxygen, esters and epoxides are susceptible to hydrolysis under the OECD test conditions (OECD Guideline 111). For these hydrolysis reactions to occur, there must be 1) an electrophylic carbon atom which is 'attacked' by oxygen; and 2) a 'leaving group' which departs from the attacked carbon atom. The hydrolysis reaction of 2,4,6-trimethylphenol (TMP) would occur by attack of water or OH^- at C1, because this is the most electropositive carbon in this molecule due to the electron withdrawing effect of the phenolic OH group. As

shown in the following figure, the product of this reaction would be TMP itself, indicating that there would be no net hydrolysis reaction.



Therefore, TMP would be hydrolytically stable under the conditions of the OECD test (OECD Guideline 111), and laboratory testing was not required.

This is supported by the following:

1. TMP is a phenol. Phenols are exempt from the OECD hydrolysis test in the Canadian new substance notification testing guideline (CEPA).
2. Phenols are not indicated as being susceptible to hydrolysis in a comprehensive review of the hydrolysis of organic compounds under environmental conditions (Mabey, 1978).
3. An online computer search of Chemical Abstracts for the TMP CAS RN (527-60-6) revealed 1360 citations. Refinement of these citations with the keywords 'hydrolysis' or 'stability' revealed 80 citations. None of these citation titles indicated the hydrolysis of TMP.

In summary, TMP was classified as hydrolytically stable under the OECD Guideline 111 test conditions because it is a phenol, which cannot undergo a net hydrolysis reaction. This is supported by the fact that phenols are exempt from the OECD hydrolysis test in the Canadian new substance notification testing guideline (CEPA).

Reference: Reimer, G.J. (2001). Unpublished report no. 11201 1513 entitled "Physical/chemical property of 2,4,6-Trimethylphenol (TMP; Mesitol); CAS RN 527-60-6, Hydrolytic stability (OECD 111) – Expert Statement", dated December 19, 2001 for General Electric Company, Pittsfield, MA, USA; from Reimer Analytical & Associates Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

8.0 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS, INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

8.1 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

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Syracuse Research Corporation. 2000. User's Guide for Estimation Programs Interface for Windows, Version 3, Syracuse Research Corporation. North Syracuse, New York.

9.0 BIODEGRADATION

Type:	Aerobic [X]; Anaerobic [ ]
Inoculum:	Not specified
Concentration of the chemical:	Not specified
Medium:	Water [ ]; Water-sediment [ ]; Soil [X]; Sewage treatment [ ]
Contact time:	28 days
Degradation:	Slowly biodegraded
Results:	Readily biodeg. [ ]; Inherently biodeg. [ ]; Under test condition no biodegradation observed [ ], Other [X]
Kinetic of test substance:	(e.g. Zahn-Wellens-Test): Theoretical BOD = 7% in 28 days
Control substance:	Not specified
Kinetic of control substance:	Not specified
Degradation Products:	Not specified
Method (Year):	Other (1992): Japanese MITI test believed to be similar to OECD 301C.
GLP:	Yes [ ] No [ ] ? [X]
Test substance:	2,4,6-Trimethylphenol (CAS RN 527-60-6) Purity and source not specified
Remarks:	Study details are not available
Reference:	Author Unknown (1992). Chemicals Inspection and Testing Institute. Japan Industry Ecology – Toxicology and Information Center. ISBN 4-89074-101-1.
Reliability:	(Klimisch Code 3) Not reliable. Missing study details, such as source and concentration of inoculum, pre-acclimation of the test material, initial concentration of the test material, temperature of incubation, and analytical methods. Documentation insufficient for assessment of validity.

ECOTOXICOLOGICAL DATA

10.0 ACUTE/PROLONGED TOXICITY TO FISH

10.0.1

Type of Test:	Static [X] Semi-static [ ] Flow-through [ ] Other [ ] Open-system [ ] Closed-system [ ]
Species:	Rainbow trout (*Oncorhynchus mykiss)* (Rainbow Springs Hatchery, Thamesford, Ontario, Canada)
Exposure Period:	96 Hours
Results:	LC_{50} (96h) = 9.7 mg/L (95% CI = 3.7 –0 17.2 mg/L)
Analytical Monitoring:	Yes [X] No [ ] ? [ ]
Method:	OECD Test Guideline 203 (1992)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6); from GE Plastics, Selkirk, NY, USA; Purity: 87.72% (Impurities: O-Cresol = 0.11%; 2,6 Xylenol = 0.81%; 2,4/2,5 Xylenol =5.61%; 2,3,6-Trimethylphenol = 1.58%; 2,6 EMP = 4.03%; Unknowns = 4.17%)
Remarks:	Definitive testing was conducted by setting up a dilution series to bracket the LC_{50} calculated in a range-finding test. The dilution series was set up as a 2.2 geometric series to achieve six exposure

concentrations (0.8, 1.7, 3.8, 8.3, 18.2 and 40 mg/L; as test product) and a control. The definitive test was based on a total of 20 fish (i.e., 10 fish per replicate, in each of two replicates) exposed to each test concentration, as well as a control (100% dilution water). Mortality and abnormal behavior (e.g., erratic swimming) were recorded at 3, 24, 48, 72 and 96 hours, and any dead fish were removed. All fish were handled using a fine mesh dip net. An LC_{50} (concentration causing lethality to 50% of the organisms) was estimated based on the 96-hour mortality data. The test was deemed valid if: 1) mortality and impairment did not exceed 10% in the control; 2) constant conditions were maintained throughout the test; 3) the dissolved oxygen concentration was at least 60% of the air saturation value throughout the test; and 4) the concentration of the substance being tested was maintained (within 80% of nominal) throughout the test (if deviation from nominal was greater than 20%, the results were based on measured concentrations). The 96-hour LC_{50} was calculated using nonlinear interpolation.

Test conditions: The fish were held 14 days before initiating the test on TMP. Mortality in the stock culture was less than or equal to 5% the week prior to test initiation. The dilution water was groundwater (initial hardness approximately 260 to 280 mg/L as $CaCO_3$) from an aquifer in Aberfoyle, Ontario, Canada. The dilution waster used for testing was adjusted to approximately pH 7.0, using hydrochloric acid. Laboratory dilution water is analyzed regularly for metals, organics, and inorganic chemicals. For the definitive test, 4 liters of an 800 mg/L stock solution was prepared in two– 2-liter volumetric flasks by mixing 1.6008 g and 1.5999 g into each flask with groundwater. The two flasks containing the 800 mg/L stock solution were thoroughly homogenized prior to preparation of each test solution. All stock solutions were prepared approximately 24 hours in advance of test initiation to allow the solution to reach equilibrium. The majority of the test substance appeared to be readily soluble in water. However, fine particulates were observed in the stock solutions during both the range-finding and definitive tests.

The following is a summary of the test conditions:

Parameter	Condition
Test type	Static
Test duration	96 hours
Temperature	15 +1°C
Light quality	Ambient laboratory illumination
Light intensity	100 to 500 lux
Photoperiod	16-hour light, 8-hour dark
Feeding prior to test	Commercial trout pellets. Feeding rate = 1 to 4% wet weight of fish.
Feeding regime	None (during preceding 24 hour and during testing)
Test chamber	Glass aquaria
Water volume	15 L (# 1.0 g fish/L)
Acclimation / Health	14-day acclimation / < 5% mortality in 7 days prior to test.
Age of test organisms	Juvenile (approximately 5 ± 1 cm)
Range-finding test concentrations	0, 0.08, 0.8, 8, 80 and 800 mg/L
Number of replicate test vessels/concentration in range-finding test	1 replicate per test and control concentration
Definitive test concentrations	0, 0.8, 1.7, 3.8, 8.3, 18.2 and 40 mg/L
Number of replicate test vessels/concentration in definitive test	2 replicates per test and control concentration
Number of animals per replicate	10
Aeration	6.5 ± 1 mL/L/min. Dissolved oxygen > 60% of saturation in control.
Dilution water	Groundwater (initial hardness approximately 260-280 mg/L as CaCO3; initial pH approximately 8.3) adjusted to approximately pH 7 prior to testing
Measured water chemistry parameters	pH, dissolved oxygen, conductivity, temperature, visual observations at 0, 3, 24, 48, 72, and 96 hours.
Measured endpoints	Mortality, stressed behavior

Test concentration analysis: Samples of the control, 0.8, 8.3 and 40 mg/L exposure concentrations of the test substance were analyzed for the active ingredient, TMP, to confirm the nominal test concentrations. The average recovery of the observed TMP concentrations in spiked aqueous solutions was 96%, demonstrating acceptable method accuracy. Measured TMP concentrations were within 20% of nominal concentrations. However, in two cases, the difference between nominal and measured concentrations was 19%. Furthermore, percent differences between old and new test solutions were also greater than 20%. Based on these results, measured concentrations were used in endpoint calculations. Exposure concentrations that were not analytically verified were interpolated from the measured concentrations. All concentrations were then time-weighted (OECD, 1998b).

Following are the results of the test concentration analyses:

Nominal TMP Concentration - as Product (mg/L)	Time Collected (hours)	Measured TMP Concentration - as Active Ingredient (mg/L)	Nominal TMP Concentration - as Active Ingredient (mg/L) *	% Difference between Nominal and Measured TMP as Active Ingredient
40	0	33.47	35.09	5
8.3	0	8.67	7.28	19
0.8	0	0.8	0.70	114
0	0	<LOD	0.00	0
40	96	41.81	35.09	119
8.3	96	7.67	7.28	105
0.8	96	0.6	0.70	14
0	96	<LOD	0.00	0

*based on product containing 87.72% TMP

Results: All fish in 8.3, 18.2 and 40 mg/L concentration were immobile within 5 minutes of exposure. All fish in the 18.2 and 40 mg/L concentrations died within 3 hours of exposure. From 24 through the end of the 96-hour exposure, all surviving fish in the 8.3 mg/L concentration were immobile and/or lying on their sides with only opercular movement/gaping; and 1 to 3 fish in the 3.8 mg/L concentration were lying on their side, had loss of equilibrium, were very dark, and/or not swimming.

The test met all of the validity criteria. The 96-hour LC_{50} was determined to be 9.7 mg/L (95% confidence limits of 3.7 to 17.2 mg/L) based on time-weighted, measured and interpolated concentrations of TMP. Based on this result and the acute Ecotoxicity classification categories, TMP would be classified as "moderately toxic".

Rainbow Trout Mortalities (%)

Nominal Concentration (mg/L)	Mortality (%) after 24 hours	Mortality (%) after 96 hours
Control	0	0
0.8	0	0
1.7	0	0
3.8	0	0
8.3	25	30
18.2	100	100
40	100	100

Reference: Novak, L. (2002). Unpublished report no. 20000865/20002536 entitled "Ecotoxicological evaluation of 2,4,6-Trimethylphenol (TMP, CAS RN 527-60-6): Acute toxicity to rainbow trout", dated December 2002 for General Electric Company, Pittsfield, MA, USA; from ESG International Inc., Guelph, Ontario, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

11.0 TOXICITY TO AQUATIC PLANTS (E.G. ALGAE)

11.0.1

Species:	Green algae (*Selenastrum capricornutum*)
End-point:	Biomass [X] Growth rate [X] Other [ ]
Exposure Period:	96 Hours
Results:	Based on the measured, interpolated and time-weighted concentrations: Cell Number: EC_{50} (72h) = 2.54 mg/L (95% CI = 2.00 – 2.86 mg/L) EC_{50} (96h) = 3.05 mg/L (95% CI = 2.81 – 3.28 mg/L) NOEC (72h) = 0.55 mg/L LOEC (72h) = 1.61 mg/L NOEC (96h) = 1.61 mg/L LOEC (96h) = 3.43 mg/L Growth: EC_{50} (72h) = 5.59 mg/L (95% CI = 5.15 – 6.07 mg/L) EC_{50} (96h) = 6.03 mg/L (95% CI = 5.38 – 6.58 mg/L) NOEC (72h) = 1.61 mg/L LOEC (72h) = 3.43 mg/L NOEC (96h) = 1.61mg/L LOEC (96h) = 3.43 mg/L Biomass (area under the curve): EC_{50} (72h) = 2.54 mg/L (95% CI = 2.16 – 2.89 mg/L) EC_{50} (96h) = 2.73 mg/L (95% CI = 2.37 – 2.98 mg/L) NOEC (72h) = 0.55 mg/L LOEC (72h) = 1.61 mg/L NOEC (96h) = 0.55 mg/L LOEC (96h) = 1.61 mg/L
Analytical Monitoring:	Yes [X] No [ ] ? [ ]
Method:	OECD Test Guideline 201 (1984); and US EPA OPPTS 850.5400 (1996)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6); from GE Plastics, Selkirk, NY, USA; Purity: 87.72% (Impurities: O-Cresol = 0.11%; 2,6 Xylenol = 0.81%; 2,4/2,5 Xylenol =5.61%; 2,3,6-Trimethylphenol = 1.58%; 2,6 EMP = 4.03%; Unknowns = 4.17%)
Remarks:	Definitive testing was conducted by setting up a dilution series to bracket the EC_{50} calculated in a range-finding test. The definitive 96-hour static EC_{50} test was conducted with nominal concentrations of 0.82, 2.05, 5.12, 12.8, 32.0 and 80.0 mg/L (a 2.5 dilution series, as test product), plus controls. After 96 hours of growth, the pH was measured in pooled samples from each concentration. Changes in cell development or appearance, such as cell clumping, cell morphology, cell color, cell shape, and cell size were reported. Test conditions: The test was initiated with exponentially growing cells (3 to 7 days old) from in-house cultures maintained at 24 ± 1°C under continuous light (4 ± 10% kLux). The cultures were grown under axenic conditions and subcultered into fresh medium twice weekly.

The following is a summary of the test conditions:

Parameter	Conditions
Test species	Uni-algal cultures of *Selenastrum capricornutum* Printz 1913 (original source UTCC #37)
Duration of test	96 hours
Culture medium	Algal growth medium (Environment Canada, 1992[1])
Testing medium	Algal growth medium (Environment Canada, 1992)
Incubation chamber	Incubated in a growth chamber
Temperature	24 ± 1°C
Light quality	Cool-white fluorescent
Light intensity	Measured at the surface of the liquid in the flasks. 4 ± 10% kLux for culturing; 8 ± 20% kLux for testing
Photoperiod	Continuous (24 hours)
Test vessel size	Clear glass 250-mL Erlenmeyer flasks
Nutrient/test solution volume	50 mL
pH of the nutrient solution	Recommended = 7.5 ± 0.1
pH of the test solutions	Measured, but not adjusted
Age of test plants	3 – 7 days
Number of cells per test vessel	1 x 10^4 cells/mL
Range-finding test concentrations	0, 0.08, 0.8, 8, 80 and 800 mg/L
Number of replicate test vessels per concentration in range-finding test	1 test replicate per test and control concentration
Definitive test concentrations	0, 0.82, 2.05, 5.12, 12.8, 32.0 and 80.0 mg/L
Number of replicate test vessels/concentration in definitive test	4 replicates per test and control concentration
Measured water quality parameters	pH at start and end of the test in all concentrations
Measured endpoints	Cell number measured daily using a haemocytometer
Calculated endpoints	Area under the growth curve, growth rate, cell number
Statistical endpoints	72- and 96-hour EC_b50 (based on area under the growth curve), 72- and 96-hour EC_r50 (based on growth rate), 72- and 96-hour EC50 for cell number, LOEC and NOEC for all three biological endpoints
Test validity criteria	1.6 x 10^5 cells/mL after 72 hours (OECD, 1984)

[1] The liquid growth medium recommended by Environment Canada (1992) is very similar to the medium referenced by the U.S. EPA (1996) and cited in ASTM (1997). There are minor differences in concentration and the order in which the nutrients are combined, but the types of nutrients recommended are identical. The Environment Canada (1992) medium meets the nutrient requirements outlined in OECD (1984). In our laboratory, the Environment Canada (1992) growth medium has been used successfully for culturing and testing with *S. capricornutum* since August 1999.

Test concentration analysis: Samples (50 mL) were collected from the control, 0.82, 5.12, 12.8 and 80.0 mg/L test concentrations at 0 and 96 hours. At 96-hours only, the 50-mL sample was a subsample of the pooled replicates. All samples were refrigerated and shipped on dry ice in sealed 50-mL polypropylene centrifuge tubes to Reimer Analytical and Associates Inc. (RAA; Vancouver, B.C., Canada) for analysis. The samples were analyzed using high performance liquid chromatography with a diode array detector (HPLC-DAD) to determine the exposure concentrations of TMP.

Following are the results of the test concentration analyses:

Nominal TMP Concentration as Product (mg/L)	Time Collected (hours)	Measured TMP Concentration as Active Ingredient (mg/L)	Nominal TMP Concentration - as Active Ingredient (mg/L)[a]	Ratio of Measured Over Nominal (TMP as Active Ingredient)
0	0	<LOD	0	-[b]
0.82	0	0.8	0.72	1.11
5.12	0	5.1	4.49	1.14
12.8	0	12.91	11.23	1.15
80	0	80.81	70.18	1.15
0	96	<LOD	0	-[b]
0.82	96	0.36	0.72	0.50
5.12	96	2.17	4.49	0.48
12.8	96	6.3	11.23	0.56
80	96	40.25	70.18	0.57

[a] Based on test substance containing 87.72% active ingredient.
[b] Could not calculate because they were below detection limit.

Results: See the following tables:

Summary of Cell Counts, Area Under Growth Curves and Growth Rate for *Selenastrum* Definitive Test

Nominal Concentration (mg/L)	Average Cell Counts (x 10,000) 24 h	48 h	72 h	96 h	Mean Area Under Growth Curve at 96 hours (x 10,000)	Mean Growth Rate at 96 hours (x 10,000)
0	7.5	36.8	243	368	11994	0.05998
0.82	5.4	36.9	235	361	11657	0.05979
2.05	6.1	28.5	193	337	10073	0.05900
5.12	3.4	12.6	73.8	156	4242	0.05123
12.8	1.4	2.0	3.5	4.5	137	0.01403
32.0	0.6	2.1	2.0	2.1	52.4	0.00610
80.0	1.3	1.5	1.6	3.0	55.2	0.00595

Percent Inhibition of Growth Rate and Area Under the Curve after 72 and 96 hours for *Selenastrum* Definitive Test

Nominal Concentration (mg/L)	Growth Rate % Inhibition at 72 hours	Growth Rate % Inhibition at 96 hours	Area Under the Curve % Inhibition at 72 hours	Area Under the Curve % Inhibition at 96 hours
0	--	--	--	--
0.82	0.6	0.3	3.7	2.8
2.05	4.2	1.6	21.0	16.0
5.12	21.9	14.6	69.2	64.6
12.8	78.7	76.6	98.5	98.9
32.0	88.6	89.8	99.3	99.6
80.0	92.8	90.1	99.4	99.5

Reference: Roshon, R. (2002). Unpublished report no. 20000865/20002536 entitled "2,4,6-Trimethylphenol (TMP, CAS RN 527-60-6) Growth inhibition test with the freshwater green alga, *Selenastrum capricornutum* Printz (OECD 201)", dated December 2002 for General Electric Company, Pittsfield, MA, USA; from ESG International Inc., Guelph, Ontario, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

12.0 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

12.1.1 Daphnia

12.1.1.1

Type of Test: Static [X] Semi-static [ ] Flow-through [ ] Other [ ]
Open-system [ ] Closed-system [ ]

Species: *Daphnia magna*

Exposure Period: 24 Hours

Results: EC_{50} (24h) = 3.5 mg/L (95% CI = 1.6 – 8.5 mg/L)

Analytical Monitoring: Yes [X] No [ ] ? [ ]

Method: OECD Test Guideline 202 (1984): Only the first portion of the OECD test procedure (i.e. the Acute Immobilization Test and not the Reproduction Test) was conducted.

GLP: Yes [X] No [ ] ? [ ]

Test Substance: 2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6); from GE Plastics, Selkirk, NY, USA; Purity: 87.72% (Impurities: O-Cresol = 0.11%; 2,6 Xylenol = 0.81%; 2,4/2,5 Xylenol =5.61%; 2,3,6-Trimethylphenol = 1.58%; 2,6 EMP = 4.03%; Unknowns = 4.17%)

Remarks: Definitive testing was conducted by setting up a dilution series to bracket the EC_{50} calculated in a range-finding test. The dilution series was set up as a 2.2 geometric series to achieve six exposure concentrations (0.8, 1.7, 3.8, 8.3, 18.2 and 40 mg/L; as test product) and a control. The definitive test was based on a total of 20 daphnids (i.e., five (5) daphnids per replicate, in each of four (4) replicates) exposed to each test concentration, as well as a control (100% dilution water). Immobility and abnormal behavior (e.g., erratic swimming) were recorded at 24 hours. An EC_{50} (concentration causing immobility in 50% of the organisms) was estimated based on the 24-hour immobility data. The test was considered valid if immobility did not exceed 10% in the control.

Test conditions: Dilution water for culturing and testing was moderately hard groundwater from an aquifer in Aberfoyle, Ontario, Canada that was continuously and vigorously aerated. Laboratory dilution water was analyzed regularly for metals, organics and inorganic chemicals. All stock solutions and exposure concentrations were dosed as product. For the definitive test, a 40 mg/L stock solution was prepared by mixing 0.0813 g of product to 2 L of groundwater. All stock solutions were prepared approximately 24 hours in advance of test initiation to allow the solution to reach equilibrium. The majority of the test substance appeared to be readily soluble in water. However, fine particulates were observed in the stock solutions during both the range-finding and definitive tests. The control was laboratory dilution water. The 24-hour EC_{50} was calculated using nonlinear interpolation.

Following is a summary of the test conditions:

Parameter	Condition
Test type	Static
Test duration	24 hours
Temperature	20 $\pm$1^{o}C, as recorded daily with a maximum/minimum thermometer
Light quality	Ambient laboratory illumination
Light intensity	400 to 800 lux (at water surface)
Photoperiod	16-hour light, 8-hour dark
Feeding prior to test	Once/day: 1) YCT and 2) *Selenastrum* and *Chlorella algae* (3:1)
Feeding regime	None (24 hours prior to and during testing)
Test chamber	500 mL BOD bottles
Loading rate	20 mL/daphnid
Test volume	Minimum 100 mL
Source	ESG laboratory culture
Age of test organisms	First instar (<24-hours old)
Range-finding test concentrations	0, 0.08, 0.8, 8, 80 and 800 mg/L
Number of replicate test vessels per concentration in range-finding test	2 replicates per test and control concentration
Definitive test concentrations	0, 0.8, 1.7, 3.8, 8.3, 18.2 and 40 mg/L
Number of replicate test vessels per concentration in definitive test	4 replicates per test and control concentration
Number of animals per replicate	5
Aeration	None
Dilution water	Groundwater (initial hardness approximately 200 mg/L as $CaCO_3$; initial pH approximately 8.3) adjusted to approximately pH 7 prior to testing.
Measured water chemistry parameters	pH, dissolved oxygen, conductivity, temperature, visual observations at 0 and 24 hours. Dilution water hardness at 0 hours.
Measured endpoints	Immobility

Chemical analysis: At the start and end of the definitive test, samples from each replicate in the control, 0.8, 8.3 and 40 mg/L test solutions were pooled and a 50-mL sub-sample saved for analyses. All samples were refrigerated prior to shipping on dry ice in sealed 50-mL polypropylene centrifuge tubes to Reimer Analytical and

Associates Inc. (RAA; Vancouver, B.C., Canada) for analysis. The samples were analyzed using high performance liquid chromatography (HPLC) with UV detection to determine the exposure concentrations of TMP.

Results: 24-hour EC_{50} = 3.5 mg/L resulting in a classification of moderately toxic.

Following are the results of the test concentration analyses:

Nominal Concentration as Product (mg/L)	Time Collected (hours)	Measured TMP Concentration as Active Ingredient (mg/L)	Nominal TMP Concentration as Active Ingredient (mg/L) *	% Difference between Nominal and Measured TMP as Active Ingredient
0	0	<LOD	0.00	0.0
0.8	0	0.85	0.70	121
8.3	0	8.83	7.28	121
40	0	44.97	35.09	128
0	24	<LOD	0.00	0.0
0.8	24	0.78	0.70	111
8.3	24	8.22	7.28	113
40	24	40.25	35.09	115

*based on product containing 87.72% TMP

Reference: Novak, L. (2002) Unpublished report no. 20000865/20002536 entitled "Ecotoxicological Evaluation of 2,4,6-Trimethylphenol (TMP, CAS RN 527-60-6): Acute toxicity to *Daphnia magna*", dated December 2002 for General Electric Company, Pittsfield, MA, USA; from ESG International Inc., Guelph, Ontario, Canada.

Reliability: (Klimisch Code 2) Valid with restrictions. Due to analytical difficulties, this study should be considered valid, but used with care.

12.1.1.2

Type of test: static [X]; semi-static [ ]; flow-through [ ]; other [ ]; open-system [ ]; closed-system [ ]

Species: *Daphnia magna* (Straus 1820)

Exposure period: 24 hours

Results: $EC_{50\ (24h)}$ = 0.208 mmol/L (95% Confidence Limit; 0.189 to 0.227)

Analytical monitoring: Yes [ ] No [ ] ? [X]

Method: The test material was diluted with reconstituted hard water (pH 7.8-8.2; hardness 200 mg/L as $CaCO_3$) and acetone was used as a dispersent solvent (volume < 0.1 mg/L). The Daphnids were supplied by the IRCHA Laboratory and cultured in the Pasteur Institute Laboratory (Lyon, France) in 10 litre tanks with aerated hard water (7.5 $\pm$ 0.4 mg/L as Ca; 5.2 $\pm$ 0.3 mg/L as Mg; pH = 7.0). Test concentrations of 0.1, 0.35, 1, 3.5, 10, 35, 100, and 350 mg/L were used in the definitive test. A series of test tubes were filled with increasing quantities of the test material solutions and water was

	added to up to a volume of 8 mL. Five daphnids (< 72 hours of age) were then placed into each test tube and more reconstituted water was added to make the volume up to 10 mL. Concurrent control tubes contained reconstituted water and the acetone dispersent solvent. The test tubes were covered with plastic stoppers and removed from the light, and held at 20 $\pm$ 1 °C without aeration. Daphnids were not fed during the course of the experiment. Observations of test populations were carried out following 24 hours of exposure. The *daphnia* that were unable to swim within 15 seconds after gentle agitation of the water were considered to be immobilized. Dissolved oxygen, pH, and temperature were measured at the end of the test. The percentage of immobilized daphnia were recorded and plotted as a function of concentration on log-probit paper. The points obtained were fitted to a straight line and the EC_{50} was determined. The test chemical was assayed in duplicate with a minimum of three replicates the following day.
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	2,4,6-trimethylphenol (TMP; CAS RN 527-60-6); from commercial source (not identified); Purity: > 95%
Remarks:	Data was not reported for the specific number of immoblized daphnia per test concentration and the end of study measurements for dissolved oxygen, pH, and temperature.
Reference:	Devillers, J. (1988) Acute toxicity of cresols, xylenols and trimethylphenols to *Daphnia Magna* Straus 1820. *The Science of Total Environment*, 76:79-83.
Reliability:	(Klimisch Code 2) Reliable with restrictions. Acceptable, well-documented publication which meets basic scientific principles.

12.2. Other Aquatic Organisms

No studies were found.

TOXICITY

13.0 ACUTE TOXICITY

13.1 ACUTE ORAL TOXICITY

13.1.1	
Type:	LD_0 [ ] LD_{100} [ ] LD_{50} [ ] LD_{L0} [ ] Other [X]
Species/Strain:	Rat/Outbred albino (*rattus norvegicus*)
Sex:	Male and female
# Animals:	3/sex
Vehicle:	Cottonseed oil
Value:	>2000 mg/kg
Method:	Acute Oral Toxic, Acute Toxicity Class Method OECD Test Guideline 423 (1996); US EPA OPPTS 870.1100 (1998)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6); from GE Plastics, Selkirk, NY, USA; Purity: 87.72% (Impurities: O-Cresol = 0.11%;

	2,6 Xylenol = 0.81%; 2,4/2,5 Xylenol = 5.61%; 2,3,6-Trimethylphenol = 1.58%; 2,6 EMP = 4.03%; Unknowns = 4.17%)
Remarks:	Six albino rats (3/sex) weighing 201 to 221 g and 8 weeks old were administered a single oral dose of 2000 mg TMP/kg body weight. All animals were fasted the night prior to dosing. The test substance was a solid and was suspended in cottonseed oil. The dose was calculated using a concentration of 1 gm/mL. The administered volume did not exceed 1 mL/100 gm body weight. The doses were administered by means of a ball tip gavaging needle and a syringe. Following dosage, the rats were provided feed and water ad libitum and were observed for appearance, behavior, body weight and mortality for a 14-day period. All rats were sacrificed at the end of the study and examined for evidence of gross pathology. All animals gained weight and no animals died at this dose during the 14-day post-dose observation period. Piloerection was observed in all test animals for the first day of the study. All signs of piloerection were resolved by Day 3 of the study in all but one animal, which was resolved by Day 4 of the study. No unusual clinical observations were observed for the rest of the study and no unusual lesions were noted in any of the animals at necropsy. Based on the absence of mortality and the criteria of the study protocol, the test substance is defined as non-toxic at a dose of 2000 mg/kg.
Reference:	Tay, C.H. (2002). Unpublished report no. 01-7021-G1 entitled "Acute oral toxicity test acute toxic class method (OECD 423)", dated March 04, 2002 for General Electric Company, Pittsfield, MA, USA; from Toxikon Corporation, Bedford, MA, USA.
Reliability:	(Klimisch Code 1) Valid without restrictions.

13.2 ACUTE INHALATION TOXICITY

No studies were found.

13.3 ACUTE DERMAL TOXICITY

Type:	LD_0 [ ] LD_{100} [ ] LD_{50} [X] LD_{L0} [ ] Other [ ]
Species/Strain:	Rabbit (New Zealand White rabbits, *Oryctolagus cuniculus*)
Value:	> 2,000 mg/kg
Method:	OECD Test Guideline 402 (1987); US EPA OPPTS 870.1200 (1998)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6); from GE Plastics, Selkirk, NY, USA;) Purity: 87.72% (Impurities: O-Cresol = 0.11%; 2,6 Xylenol = 0.81%; 2,4/2,5 Xylenol = 5.61%; 2,3,6-Trimethylphenol = 1.58%; 2,6 EMP = 4.03%; Unknowns = 4.17%)
Remarks:	Ten rabbits (5/sex), weighing 2.02-2.26 kg and 12 weeks of age, were dosed with a single dermal application of 2000 mg TMP/kg body weight for 24 hours. TMP was moistened with water to a paste before being introduced under gauze patches, two single layers thick and applied directly to the skin (approximately 10%) of the body surface. Animals were immobilized and the patches were secured in place by wrapping the entire trunk of the animal with an impervious bandage. Test sites were secured to prevent the animals from ingesting the test substance. Following the 24-hour exposure, all animals gained weight during the 14-day post-treatment observation

period. No overt signs of systemic toxicity were evident during the course of the study and no animals died. At necropsy, there were no abnormalities or lesions noted. After removal of the TMP, necrosis was observed in six out of ten animals. The necrotic areas remained visible for the duration of the study. Slight to moderate erythema and edema was observed in the remaining four animals. All signs of erythema and edema were resolved by Day 11 of the study. Based on the absence of mortality, the LD_{50} was determined to be greater than 2000 mg/kg and the test material was classified as non-toxic.

Reference: Tay, C.H. (2002). Unpublished report no. 01-7021-G2 entitled "Acute dermal toxicity study", dated March 20, 2002 for General Electric Company, Pittsfield, MA, USA; from Toxikon Corporation, Bedford, MA, USA.

Reliability: (Klimisch Code 1) Valid without restrictions.

14.0 GENETIC TOXICITY *IN VITRO* OR *IN VIVO* (CHROMOSOMAL ABERRATIONS)

14.0.1

Type: *In vitro* mammalian chromosome aberration test

System of testing: Chinese hamster ovary (CHO) cells

Concentration: 0, 25, 50, 100, 150, 200 and 300 µg/mL (4-hr treatment w/o S9 mix)
0, 25, 50, 100, 150, 200, 300 and 400 µg/mL (20-hr treatment w/o S9 mix)
0, 12.5, 25, 50, 100, 150 and 200 µg/mL (4-hr treatment with S9 mix)

Metabolic activation: With [ ]; Without [ ]; With and Without [X]; No data [ ]

Results: Negative without metabolic activation
Positive with metabolic activation

Cytotoxicity conc: With metabolic activation: ≥ 136 µg/mL
Without metabolic activation: ≥ 408 µg/mL

Precipitation conc: With metabolic activation: 1360 µg/mL
Without metabolic activation: 1360 µg/mL

Genotoxic effects:

	+	?	-
With metabolic activation:	[X]	[ ]	[ ]
Without metabolic activation:	[ ]	[ ]	[X]

Method: OECD Test Guideline 473 (1998)

GLP: Yes [X] No [ ] ? [ ]

Test Substance: 2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6; Lot #7/31/01); from GE Plastics) Purity: 87.72% (Impurities: O-Cresol = 0.11%; 2,6 Xylenol = 0.81%; 2,4/2,5 Xylenol =5.61%; 2,3,6-Trimethylphenol = 1.58%; 2,6 EMP = 4.03%; Unknowns = 4.17%)

Remarks: Description of test procedure: A preliminary toxicity assay was performed for the purpose of selecting doses for the chromosome aberration assay and consisted of an evaluation of test article effect on cell growth. CHO cells were seeded for each treatment condition at approximately 5 x 10^5 cells/25 cm^2 flask and were incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for 16-24 hours. Treatment was carried out by refeeding the flasks with 5 mL complete medium (McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS), 100 units penicillin and 100 µg streptomycin/mL, and 2 mM L-glutamine) for the non-activated study or S9 reaction mixture (4 mL serum-free medium plus 1 mL of S9/cofactor pool) for the activated study, to which was added 50 µL dosing solution of test article in solvent or solvent alone. The

osmolality of the highest concentration of dosing solution in the treatment medium was measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. The cells were treated for 4 hours with and without S9, and continuously for 20 hours without S9. At completion of the 4-hour exposure period, the treatment medium was removed, the cells washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS), refed with 5 mL complete medium and returned to the incubator for a total of 20 hours from the initiation of treatment. At 20 hours after the initiation of treatment the cells were harvested by trypsinization and counted using a Coulter counter. The presence of test article precipitate was assessed using the unaided eye. Cell viability was determined by trypan blue dye exclusion. The cell counts and percent viability were used to determine cell growth inhibition relative to the solvent control. In the preliminary toxicity assay, the maximum dose tested was 1610 µg/mL. The test article was soluble in treatment medium at all doses tested. Selection of doses for microscopic analysis was based on toxicity (the lowest dose with at least 50% reduction in cell growth relative to the solvent control and two lower doses) in the non-activated 4-hour exposure group. Selection of doses for microscopic analysis was based on mitotic inhibition (the lowest dose with at least 50% reduction in mitotic index relative to the solvent control and two lower doses) in the S9 activated 4-hour exposure group and in the non-activated 20-hour exposure group. Based on the toxicity study, the doses chosen for the chromosome aberration assay were 0, 25, 50, 100, 150, 200 and 300 µg/mL (4-hr treatment w/o S9 mix), 0, 25, 50, 100, 150, 200, 300 and 400 µg/mL (20-hr treatment w/o S9 mix), and 0, 12.5, 25, 50, 100, 150 and 200 µg/mL (4-hr treatment with S9 mix).

For the chromosome aberration assay, CHO cells were seeded at approximately 5 x 10^5 cells/25 cm^2 flask and were incubated at 37 ± 1°C in a humidified atmosphere of 5 ± 1% CO_2 in air for 16 to 24 hours. Treatment was carried out by refeeding duplicate flasks with 5 mL complete medium (McCoy's 5A medium supplemented with 10% FBS, 100 units penicillin and 100 µg streptomycin/mL, and 2 mM L-glutamine) for the non-activated study or 5 mL S9 reaction mixture for the S9 activated study, to which was added 50 µL of dosing solution of test or control article in solvent or solvent alone. The osmolality of the highest concentration of dosing solution in the treatment medium was measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. In the non-activated study, the cells were exposed to the test article continuously for 4 or 20 hours After the exposure period for the4-hour exposure group, the treatment medium was removed, the cells washed with CMF-PBS, refed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/mL and the flasks returned to the incubator until cell collection. In the S9 activated study, the cells were exposed for 4 hours. After the exposure period, the treatment medium was removed, the cells washed with CMF-PBS, refed with complete medium and returned to

the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/mL and the flasks were returned to the incubator until cell collection. Two hours after the addition of Colcemid®, metaphase cells were harvested for both the non-activated and S9 activated studies by trypsinization. Cells were collected approximately 20 hours after initiation of treatment. The cells were collected by centrifugation at approximately 800 rpm for 5 minutes. The cell pellet was resuspended in 2-4 mL 0.075 M potassium chloride (KCl) and allowed to stand at room temperature for 4-8 minutes. The cells were collected by centrifugation, the supernatant aspirated and the cells fixed with two washes of approximately 2 mL Carnoy's fixative (methanol:glacial acetic acid, 3:1, v/v). The cells were stored overnight or longer in fixative at approximately 2-8°C. To prepare slides, the fixed cells were centrifuged at approximately 800 rpm for 5 minutes, the supernatant was aspirated, and 1 mL fresh fixative was added. After additional centrifugation (at approximately 800 rpm for 5 minutes) the supernatant fluid was decanted and the cells resuspended to opalescence in fresh fixative. A sufficient amount of cell suspension was dropped onto the center of a glass slide and allowed to air dry. The dried slides were stained with 5% Giemsa, air dried and permanently mounted.

Evaluation of metaphase cells: Slides were coded using random numbers by an individual not involved with the scoring process. To ensure that a sufficient number of metaphase cells were present on the slides, the percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. Metaphase cells with 20 ± 2 centromeres were examined under oil immersion without prior knowledge of treatment groups. Initially, the non-activated and S9 activated 4-hour exposure groups were evaluated for chromosome aberrations and since a negative result was obtained in the non-activated 4-hour exposure group, the non-activated 20-hour continuous exposure group was then also evaluated for chromosome aberrations. When possible, a minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations. The number of metaphase spreads that were examined and scored per duplicate flask may have been reduced if the percentage of aberrant cells reaches a statistically significant level before 100 cells are scored. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells (≥10 aberrations) were also recorded. Chromatid and isochromatid gaps were recorded but not included in the analysis. The XY coordinates for each cell with chromosomal aberrations were recorded using a calibrated microscope stage. Polyploid and endoreduplicated cells

were evaluated from each treatment flask per 100 metaphase cells scored.

Evaluation of test results: The toxic effects of treatment were based upon cell growth inhibition relative to the solvent-treated control and are presented for the toxicity and aberration studies. The number and types of aberrations found, the percentage of structurally and numerically damaged cells (percent aberrant cells) in the total population of cells examined, and the mean aberrations per cell were calculated and reported for each group. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell. Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test. Fisher's test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's test at any test article dose level, the Cochran-Armitage test was used to measure dose-responsiveness. All conclusions were based on sound scientific basis; however, as a guide to interpretation of the data, the test article was considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). However, values that are statistically significant but do not exceed the range of historic solvent controls may be judged as not biologically significant. Test articles not demonstrating a statistically significant increase in aberrations will be concluded to be negative. Negative results with metabolic activation may need to be confirmed on a case-by-case basis. In those cases where confirmation of negative results is not necessary, justification will be provided.

Criteria for evaluating results: The frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control.

Plates/test: Samples were run in duplicate, with and without metabolic activation.

Activation system: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice.

Negative and Positive controls: Mitomycin C (MMC) was used as the positive control in the non-activated study at final concentrations of 0.1 and 0.2 µg/mL. Cyclophosphamide (CP) was used as the positive control in the S9 activated study at final concentrations of 10 and 20 µg/mL. The solvent vehicle for the test article, dimethyl sulfoxide (DMSO), was used as the solvent control at the same concentration as that found in the test article-treated groups.

Results: In the chromosome aberration assay, the test article was soluble in treatment medium at all doses tested. The osmolality in the treatment medium of the highest concentration tested (400 µg/mL), was 386 mmol/kg. The osmolality of the solvent (DMSO) in treatment medium was 426 mmol/kg. The pH of the highest

concentration of test article in treatment medium was approximately 6.5.

4-hour harvest without metabolic activation: Toxicity TMP (cell growth inhibition relative to the solvent control) in CHO cells when treated for 4 hours in the absence of S9 activation was 63% at 300 µg/mL, the highest test concentration evaluated for chromosome aberrations. The mitotic index at the highest dose level evaluated for chromosome aberrations, 300 µg/mL, was 40% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 50, 150 and 300 µg/mL. The percentage of cells with structural aberrations in the test article-treated groups was significantly increased above that of the solvent control at dose level 300 µg/mL ($p \leq 0.05$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p < 0.05$). However, the percentage of cells with structural aberrations in the test article-treated group (3.0%) was within the historical solvent control range of 0.0% to 5.5%. Therefore, the increase in structural aberrations is not considered to be biologically significant. The percentage of cells with numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (11.5%) was statistically significant.

4-hour harvest with metabolic activation: Toxicity of TMP (cell growth inhibition relative to the solvent control) in CHO cells when treated for 4 hours in the presence of S9 activation was 35% at 200 µg/mL, the highest test concentration evaluated for chromosome aberrations. The mitotic index at the highest dose level evaluated for chromosome aberrations, 200 µg/mL, was 61% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 50, 100 and 200 µg/mL. The percentage of cells with structural aberrations in the test article-treated groups was statistically increased above that of the solvent control at dose levels 100 and 200 µg/mL ($p \leq 0.05$ at dose level 100 µg/mL and $p \leq 0.01$ at dose level 200 µg/mL, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response ($p < 0.05$). The percentage of cells with numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the CP (positive control) treatment group (38.0%) was statistically significant.

20-hour harvest without metabolic activation: In the absence of a positive response in the non-activated 4 hour exposure group, slides from the non-activated 20 hour exposure group were evaluated for chromosome aberrations. Toxicity of TMP (cell growth inhibition relative to the solvent control) was 40% at 100 µg/mL, the highest test concentration evaluated for chromosome aberrations in the non-activated 20 hour continuous exposure group. The mitotic index at the highest dose level evaluated for chromosome aberrations, 100 µg/mL, was 56% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 25, 50 and 100 µg/mL. The percentage of cells with structural and numerical aberrations in the test article-treated groups was not significantly

increased above that of the solvent control at any dose level ($p>0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (11.0%) was statistically significant.

Overall Conclusion: The positive and solvent controls fulfilled the requirements for a valid test. Under the conditions of the assay, TMP was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in the non-activated test system. TMP was concluded to be positive for the induction of structural chromosome aberrations in CHO cells in the S9 activated test system with a lowest effective dose level of 100 µg/mL and a no-effect dose level of 50 µg/mL; and TMP was concluded to be negative for the induction of numerical chromosome aberrations in CHO cells in the S9 activated test system.

Summary of Test Results

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean ± SD)	Cells with Numerical Aberrations (%)	Cells with Structural Aberrations (%)
Vehicle (DMSO)	-	4	8.2	200	0.000 ± 0.000	0.0	0.0
2,4,6-Trimethyl Phenol (TMP)							
50	-	4	7.0	200	0.000 ± 0.000	1.5	0.0
150	-	4	5.9	200	0.000 ± 0.000	1.0	0.0
300	-	4	4.9	200	0.045 ± 0.289	1.5	3.0*
Positive control (MMC) 0.2	-	4	6.1	200	0.125 ± 0.361	0.0	11.5**
Vehicle (DMSO)	+	4	8.7	200	0.03520 ± 0.184	3.0	3.5
2,4,6-Trimethyl Phenol (TMP)							
50	+	4	8.2	200	0.045 ± 0.231	4.5	4.0
100	+	4	7.4	200	0.095 ± 0.341	4.5	8.0*
200	+	4	3.4	200	0.210 ± 0.497	5.0	17.0**
Positive control (CP) 10	+	4	4.1	100	0.560 ± 0.845	5.0[β]	29.5**
Vehicle (DMSO)	-	20	6.2	200	0.000 ± 0.000	0.0	0.0
2,4,6-Trimethyl Phenol (TMP)							
25	-	20	5.3	200	0.000 ± 0.000	0.0	0.0
50	-	20	4.5	200	0.005 ± 0.071	0.0	0.5
100	-	20	2.7	200	0.000 ± 0.000	2.0	0.0
Positive control (MMC) 0.1	-	20	5.6	200	0.170 ± 0.790	0.0	11.0**

Treatment: Cells from both the 4-hour and 20 hour treatment regimens were harvested 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, $p\leq0.05$; **, $p\leq0.01$; using the Fisher's exact test.

[β] Numerical aberrations are out of 200 cells scored.

Reference: Gudi, R. and C. Brown. (2002). Unpublished report no AA52LV.331.BTL entitled "In vitro mammalian chromosome aberration test" dated November 25, 2001 for General Electric Company, Pittsfield, MA, USA; from BioReliance Corp., Rockville, MD, USA.

Reliability: (Klimisch Code 1) Valid without restrictions.

14.0.2

Type: *In vivo* mammalian micronucleus test

Species/Strain: Mouse/ICR

Sex: Male [ ]; Female [ ]; Male/Female [X]

Concentration: 0, 500, 1000, 1200, 1400 and 1600 mg/kg

Route of administration: Intraperitoneal injection

Genotoxic effects: Negative

Method: OECD Test Guideline 474 (1998)

GLP: Yes [X] No [ ] ? [ ]

Test Substance: 2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6; Lot #7/31/01); from GE Plastics) Purity: 87.72% (Impurities: O-Cresol = 0.11%; 2,6 Xylenol = 0.81%; 2,4/2,5 Xylenol =5.61%; 2,3,6-Trimethylphenol = 1.58%; 2,6 EMP = 4.03%; Unknowns = 4.17%)

Remarks: Description of test procedure: A preliminary toxicity study was conducted at dose levels of 500, 1000, 1200, 1400 and 1600 mg/kg with five male and five female mice per group. Based on the results of the preliminary toxicity study, the high dose for the definitive micronucleus test was set at 500 mg/kg, the maximum tolerated dose. In the definitive micronucleus study, mice were assigned to seven experimental groups of five males and five females. An additional group of five males and five females was designated as a replacement group to be used in the event of mortality prior to the scheduled sacrifice time, and was dosed with the high dose. Each mouse was given a sequential number and identified by an ear tag. The study design was as follows:

TMP (mg/kg)	Number of Mice Per Sex Dosed	Number of Mice Per Sex Used for Bone Marrow Collection After Dose Administration	
		24 hr	48 hr
0 (Vehicle Control)	10	5	5
125	5	5	0
250	5	5	0
500	15*	5	5
Positive Control: CP (50 mg/kg)	5	5	0

* Includes 5 replacement animals per sex to ensure the availability of five animals for micronucleus analysis

The vehicle for TMP was corn oil. The test article-vehicle mixture, the vehicle alone, or the positive control (cyclophosphamide monohydrate) was administered by a single intraperitoneal injection at a dose volume of 20 mL/kg body weight. At the scheduled sacrifice times, five mice per sex per treatment were sacrificed by CO_2 asphyxiation. Immediately following sacrifice, the femurs were distally exposed, cut just above the knee, and the bone marrow was

aspirated into a syringe containing fetal bovine serum. The bone marrow cells were transferred to a capped centrifuge tube containing approximately 1 mL fetal bovine serum. The bone marrow cells were pelleted by centrifugation at approximately 100 x g for five minutes and the supernatant was drawn off, leaving a small amount of serum with the remaining cell pellet. The cells were resuspended by aspiration with a capillary pipet and a small drop of bone marrow suspension was spread onto a clean glass slide. Two slides were prepared from each mouse. The slides were fixed in methanol, stained with May-Gruenwald-Giemsa and permanently mounted. Bone marrow cells, polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) were analyzed for the presence of micronuclei. Using medium magnification (10 x 40), an area of acceptable quality was selected such that the cells were well spread and stained. Using oil immersion (10 x 100), 2000 polychromatic erythrocytes per animal were scored for the presence of micronuclei. The number of MNCEs in the field of 2000 PCEs was enumerated for each animal in order to assess the quality of the differential staining procedure. The proportion of PCEs to total erythrocytes was also recorded per 1000 erythrocytes.

Evaluation of Test Results: The incidence of MPCEs per 2000 PCEs was determined for each mouse and treatment group. Statistical significance was determined using the Kastenbaum-Bowman tables which are based on the binomial distribution (Kastenbaum and Bowman, 1970). All analyses were performed separately for each sex and sampling time. In order to quantify the proliferation state of the bone marrow as an indicator of bone marrow toxicity, the proportion of PCEs to total erythrocytes was determined for each animal and treatment group. All conclusions were based on sound scientific judgment; however, as a guide to interpretation of the data, the test article was considered to induce a positive response if a dose-responsive increase in MPCEs was observed and one or more doses were statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables) at any sampling time. However, values that were statistically significant but did not exceed the range of historical negative or vehicle controls were judged as not biologically significant.

Criteria for a valid test: The mean incidence of MPCEs must not exceed 5/1000 PCEs (0.5%) in the vehicle control. The incidence of MPCEs in the positive control group must be significantly increased relative to the vehicle control group ($p \leq 0.05$, Kastenbaum-Bowman Tables).

Results: TMP was soluble in corn oil at 100 mg/mL, the maximum concentration tested in the study. No mortality occurred at any dose level during the course of the study. Clinical signs following dose administration included: piloerection in male and female mice at all doses and lethargy in males and females at 250 mg/kg. In addition, prostration and irregular breathing were observed in males and females at 500 mg/kg. Bone marrow cells (polychromatic erythrocytes, PCEs and normochromatic erythrocytes, NCEs), collected 24 and 48 hours after treatment were examined microscopically for presence of micronuclei (MPCEs or MNCEs). No appreciable reductions in the ratio of PCEs to total erythrocytes

was observed in the test article-treated groups relative to the vehicle control groups suggesting that the test article did not inhibit erythropoiesis. The number of MPCEs per 1000 PCEs in test article-treated groups was not statistically increased relative to the respective vehicle controls in either male or female mice, regardless of dose level or bone marrow collection time ($p>0.05$, Kastenbaum-Bowman Tables). In addition, no appreciable increase in the number of MNCEs in the field of 2000 PCEs per animal was found indicating that an optimal differential staining was achieved. CP induced a significant increase in MPCEs in both male and female mice ($p\leq0.05$, Kastenbaum-Bowman Tables). The negative and positive controls were consistent with the historical control data, indicating that there was no problem with the test system or the quality of the test. Following is a summary of the results:

Summary of Bone Marrow Micronucleus Analysis After a Single Dose of 2,4,6-Trimethyl Phenol (TMP, CAS RN 527-60-6) in ICR Mice

						Micronucleated Polychromatic Erythrocytes	
Treatment (20 mL/kg)	**Sex**	**Time (hr)**	**Number of Mice**	**PCE/Total Erythrocytes (Mean +/- SD)**	**Change from Control (%)**	**Number per 1000 PCEs (Mean +/- SD)**	**Number per PCEs Scored[1]**
Corn Oil							
	M	24	5	0.490 ± 0.04	---	0.2 ± 0.27	2 / 10000
	F	24	5	0.482 ± 0.05	---	0.6 ± 0.22	6 / 10000
TMP							
125 mg/kg	M	24	5	0.457 ± 0.05	-7	0.5 ± 0.00	5 / 10000
	F	24	5	0.439 ± 0.01	-9	0.2 ± 0.27	2 / 10000
250 mg/kg	M	24	5	0.430 ± 0.01	-12	0.6 ± 0.42	6 / 10000
	F	24	5	0.451 ± 0.03	-6	0.3 ± 0.27	3 / 10000
500 mg/kg	M	24	5	0.461 ± 0.04	-6	0.4 ± 0.22	4 / 10000
	F	24	5	0.445 ± 0.07	-8	0.3 ± 0.27	3 / 10000
CP							
50 mg/kg	M	24	5	0.345 ± 0.03	-30	15.7 ± 4.44	*157 / 10000
	F	24	5	0.355 ± 0.05	-26	14.0 ± 2.03	*140 / 10000
Corn Oil							
	M	48	5	0.456 ± 0.04	---	0.3 ± 0.27	3 / 10000
	F	48	5	0.478 ± 0.06	---	0.5 ± 0.00	5 / 10000
TMP							
500 mg/kg	M	48	5	0.463 ± 0.02	2	0.4 ± 0.22	4 / 10000
	F	48	5	0.439 ± 0.04	-8	0.1 ± 0.22	1 / 10000

Reference:	Gudi, R. and L. Krsmanovic (2002) Unpublished report no. AA52LV.123.BTL entitled "Mammalian Erythrocyte Micronucleus Test; 2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6)", dated December 4, 2002 for General Electric Company, Pittsfield, MA, USA; from BioReliance, Rockville, MD, USA.
Reliability:	(Klimisch Code 1) Valid without restriction.

15.0 GENETIC TOXICITY *IN VITRO*

No studies were found.

15.1 BACTERIAL TEST

Type:	Bacterial reverse mutation assay (Ames test)
System of testing:	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537
Concentrations:	0, 0.03, 0.3, 3, and 30 μmol/plate
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results:	Negative
Cytotoxicity conc.:	With metabolic activation: 30 μmol/plate Without metabolic activation: 30 μmol/plate
Precipitation conc.:	> 30 μmol/plate
Genotoxic effects:	With metabolic activation: positive [ ]; ambiguous [ ]; Negative [X] Without metabolic activation: positive [ ]; ambiguous [ ]; Negative [X]
Method:	Based on Ames et al. (1975) *Mut. Res*., 31:347. The protocol is comparable to OECD Test Guideline 471 *Description of test procedure*: The *Salmonella typhimurium* strains were supplied by Dr. Bruce N. Ames, University of California, Berkeley, USA. Spectroscopic-grade ethanol was used to prepare the test substance solution. Cultures were grown in Oxoid nutrient broth no. 2. Revertants were scored on glucose minimal salts medium supplemented with 0.05 μmol biotin. Plates used for viable counts contained 10 μmol histidine and 0.05 μmol biotin. The experiments were conducted as described by Ames et al. The positive control used that did not require metabolic activation was N-methyl-N'-nitro-N-nitrosoquanidin cyclophosphamide (concentration per plate not specified). The positive control requiring metabolic activation was 2-aminoanthracene. Both Aroclor 1254 and 3-methylcholanthrene (MCA), suspended in corn oil, were used as metabolic-inducing agents. Aroclor 1254 was administered as a single dose to male Sprague-Dawley rats 5 days prior to sacrifice, while 20 mg/kg of 3-MCA was administered for 3 days prior to sacrifice. The S-9 fraction was prepared by centrifugation of the liver homogenate at 9000 g for 10 minutes, and aliquots were then stored at -70° C. Each one mL of the S-9 mix contained 100 μmol sodium phosphate buffer, 8 μmol $MgCl_2$, 33 μmol KCl, 5 μmol glucose 6-phosphate, 4 μmol NADP, and 0.03-0.1 mL of S-9.
GLP:	Yes [ ] No [X] ? [ ]
Test substance:	2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6) Commercial; Purity: > 97% (Source not specified but purity was analytically confirmed)

Remarks:	The number of revertants/plate produced by treatment of the bacteria with the test substance at all concentrations and in all tester strains was reported to be less than or approximately equal to the number of revertants in the vehicle-treated negative control group, with and without metabolic activation. The test material was therefore concluded to not be mutagenic in this assay. Specific test article data were not shown. *Criteria for evaluating results*: No specified *Plates/test*: Not specified *Activation system*: The S-9 fraction from rat liver was induced with Aroclor 1254 or 3-MCA and prepared just prior to use (see details above). *Media*: Aqueous agar solution
Reference:	Florin, I. et al. (1980). Screening of tobacco smoke constituents for mutagenicity using the Ames test. *Toxicology*, 18:219-232.
Reliability:	(Klimisch Code 2) Valid with restrictions. Acceptable study report that meets basic scientific principles. Actual test data were not shown in the publication and the specific criteria for evaluating results was not described.

15.1.2

Additional References for Genetic Toxicity *In Vitro*:

A negative Ames assay using strains TA 98 and TA100 (conducted by Beauchamp and Shelby, Environmental Mutagen Information Center, Oak Ridge National Laboratory, Oak Ridge, TN) was reported in a publication by Epler et al., (*Environmental Health Perspectives*, 30:179-184, 1979). Specific study details were not provided.

15.2 NON-BACTERIAL *IN VITRO* TEST (MAMMALIAN CELLS)

Type:	*In vitro* mammalian cell gene mutation test (Mouse lymphoma assay)
System of testing:	Mouse lymphoma L5178Y cells
Concentration:	0, 10, 30, 40, 50 and 100 μg/mL with activation 0, 50, 75, 100, 125 and 150 μg/mL without activation
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results:	Equivocal in the absence of metabolic activation with 4- and 24-hour exposures and Negative in the presence of metabolic activation.
Cytotoxicity conc.:	With metabolic activation: ≥ 30 μg/mL Without metabolic activation: ≥ 75 μg/mL
Precipitation conc.:	1360 μg/mL (at preliminary toxicity assay)

Genotoxic effects:

	+	?	-
With metabolic activation:	[ ]	[ ]	[X]
Without metabolic activation:	[ ]	[X]	[ ]

Method:	OECD Test Guideline 476 (1998)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	2,4,6-Trimethylphenol (TMP; CAS RN 527-60-6; from General Electric Plastics): Purity: 87.72% (Impurities: O-Cresol = 0.11%; 2,6 Xylenol = 0.81%; 2,4/2,5 Xylenol =5.61%; 2,3,6-Trimethylphenol = 1.58%; 2,6 EMP = 4.03%; Unknowns = 4.17%)

Remarks:	Description of test procedure: The preliminary toxicity assay was used to establish the optimal dose levels for the mutagenesis assay. L5178Y cells were exposed to the solvent alone and nine concentrations of test article ranging from 0.15 to 1360 μg/mL in both the absence and presence of S9-activation with a 4-hour exposure and without activation with a 24-hour exposure. Cell population density was determined 24 and 48 hours after the initial exposure to the test article. The cultures were adjusted to $3x10^5$ cells/mL after 24 hours only. Cultures with less than $3x10^5$ cells/mL were not adjusted. Toxicity was measured as suspension growth of the treated cultures relative to the growth of the solvent control cultures. Treatment was carried out in conical tubes by combining 6 x 10^6 L5178Y/$TK^{+/-}$ cells, F_0P medium or S9 activation mixture and 100 μL dosing solution of test or control article in solvent or solvent alone in a total volume of 10 mL. At least eight concentrations of test article were tested in duplicate. The positive controls were treated with MMS (at final concentrations in treatment medium of 10 and 20 μg/mL with a 4-hour exposure or 2.5 and 5.0 μg/mL with a 24-hour exposure) and 7,12-DMBA (at final concentrations in treatment medium of 5.0 and 7.5 μg/mL). Treatment tubes were gassed with 5 ± 1% CO_2 in air, capped tightly, and incubated with mechanical mixing for 4 or 24 hours at 37 ± 1°C. The preparation and addition of the test article dosing solutions were carried out under amber lighting and the cells were incubated in the dark during the exposure period. After the treatment period, the cells were washed twice with F_0P or F_0P supplemented with 10% horse serum, 2 mM L-glutamine, 100 U penicillin/mL and 100 μg streptomycin/mL ($F_{10}P$). After the second wash, the cells were resuspended in $F_{10}P$, gassed with 5 ± 1% CO_2 in air and placed on the roller drum apparatus at 37 ± 1°C. Expression of the mutant phenotype: For expression of the mutant phenotype, The cultures were counted using an electronic cell counter and adjusted to $3x10^5$ cells/mL at approximately 24 and 48 hours after treatment in 20 and 10 mL total volume, respectively. Cultures with less than $3x10^5$ cells/mL were not adjusted. For expression of the $TK^{-/-}$ cells, cells were placed in cloning medium (C.M.) containing 0.23% dissolved granulated agar in F_0P plus 20% horse serum. Two flasks per culture to be cloned were labeled with the test article concentration, activation condition, and either TFT (trifluorothymidine, the selective agent) or VC (viable count). Each flask was prewarmed to 37 ± 1ºC, filled with 100 mL C.M., and placed in an incubator shaker at 37 ± 1°C until used. The cells were then diluted in C.M. to concentration of $3x10^6$ cells/100/mL C.M. for the TFT flask and 600 cells/100mL C.M. for the VC flask. After the dilution, 1.0 mL of stock solution of TFT was added to the TFT flask (final concentration of 3 μg/mL) and both this flask and the VC flask were placed on the shaker at 125 rpm and 37 ± 1°C. After 15 minutes, the flasks were removed and the cell suspension was divided equally into each of the three appropriately labelled petri dishes. To accelerate the gelling process, the plates were placed in cold storage (approximately 4°C) for approximately 30 minutes. The plates were then incubated at 37 ± 1°C in a humidified 5 ± 1% CO_2 atmosphere for 10-14 days.

Scoring procedures: After the incubation period, the VC plates were counted for the total number of colonies per plate and the total relative growth determined. The TFT-resistant colonies were then counted for each culture with ≥20% total relative growth (including at least one concentration with ≥10% but ≤20% total growth). The diameters of the TFT-resistant colonies for the positive and solvent controls and, in the case of a positive response, the test article-treated cultures were determined over a range of approximately 0.2 to 1.1 mm.

Evaluation of results: The cytotoxic effects of each treatment condition were expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency (number of mutants per 10^6 surviving cells) was determined by dividing the average number of colonies in the three TFT plates by the average number of colonies in the three corresponding VC plates and multiplying by the dilution factor ($2x10^{-4}$) then multiplying by 10^6. In evaluation of the data, increases in mutant frequencies that occurred only at highly toxic concentrations (i.e., less than 10% total growth) were not considered biologically relevant. All conclusions were based on sound scientific judgment; however, the following criteria are presented as a guide to interpretation of the data: (1) A result was considered positive if a concentration-related increase in mutant frequency was observed and one or more dose levels with 10% or greater total growth exhibited mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level. (2) A result was considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over the background level. (3) A result was considered negative if the mutant frequency in treated cultures was fewer than 55 mutants per 10^6 clonable cells over the background level.

Criteria for evaluating results: For the negative control, the spontaneous mutant frequency of the cultures must be within 20 to 100 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent control group must be greater than 50%. For positive controls, at least one concentration of each positive control must exhibit mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies. For the TMP cultures, a minimum of four analyzable concentrations with mutant frequency data was required.

Plates/test: Samples were run in duplicate, with and without metabolic activation.

Activation system: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice.

Negative and Positive controls: Dimethyl sulfoxide (DMSO) was the vehicle for the test article and served as the negative control. TMP was soluble in DMSO at 500 mg/mL, the maximum concentration tested. Methyl methanesulfonate (MMS) was used as the positive control for the non-activated test system at a stock concentration of 1000 and 2000 µg/mL with a 4-hour exposure or 250 and 500 µg/mL

with a 24-hour exposure. 7,12-Dimethyl-benz(a)anthracene (7,12-DMBA) was used as the positive control for the S9-activated test system at stock concentrations of 500 and 750 µg/mL.
Results: The maximum dose tested in the preliminary toxicity assay was 1360 µg/mL. Visible precipitate was present at 1360 µg/mL in treatment medium. No visible precipitate was present at concentrations of ≤500 µg/mL in treatment medium. The osmolality of the solvent control was 464 mmol/kg and the osmolality of the highest soluble dose, 500 µg/mL, was 447 mmol/kg. Suspension growth relative to the solvent controls was 0% at 500 µg/mL without activation with 4- and 24-hour exposures and 0% at 150 µg/mL with S9 activation. Based on the results of the toxicity test, the doses chosen for the mutagenesis assay ranged from 15 to 500 µg/mL for non-activated cultures with a 4-hour exposure, 5.0 to 150 µg/mL for S9-activated cultures with a 4-hour exposure, and 5.0 to 200 µg/mL for non-activated cultures with a 24-hour exposure.
Results for cultures treated for four hours (initial assay): No visible precipitate was present at any dose level in treatment medium. One cloned non-activated culture (treated with 125 µg/mL) exhibited a mutant frequency of 100 mutants per 10^6 clonable cells over that of the solvent control. One S9-activated culture (treated with 40 µg/mL) and five non-activated cloned cultures (treated with 75, 100, 125, and 150 µg/mL) exhibited mutant frequencies that were between 56 and 98 mutants per 10^6 clonable cells over that of the solvent control. A dose-response trend was not observed in the non-activated or S9-activated systems. The total growths ranged from 13% to 67% for the non-activated cultures at concentrations of 50 to 150 µg/mL and 12% to 102% for the S9-activated cultures at concentrations of 10 to 100 µg/mL. The results of the initial 4-hour exposure assay were equivocal in the absence of S9 activation and negative in the presence of S9 activation. Because no unique metabolic requirements were known about the test article, only an extended treatment assay was performed in the absence of S9 for a 24-hour exposure period.
Results for cultures treated for 24 hours (extended treatment assay): No visible precipitate was present at any dose level in treatment medium. Cultures treated with concentrations of 50, 75, 100, 125, and 150 µg/mL were cloned and produced a range in suspension growth of 31% to 70%.
One cloned culture (treated with 100 µg/mL) exhibited a mutant frequency of 115 mutants per 10^6 clonable cells over that of the solvent control. Two cloned cultures (treated with 75 and 100 µg/mL) exhibited mutant frequencies that were between 57 and 61 mutants per 10^6 clonable cells over that of the solvent control. A dose-response trend was not observed. The total growths ranged from 28% to 70% at concentrations of 50 to 150 µg/mL.
The TFT-resistant colonies for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants.

Cloning Data for L5178Y/TK$^{+/-}$ Mouse Lymphoma Cells Treated with TMP in the Absence of Exogenous Metabolic Activation Initial Assay (4-hour exposure)

Dose Level (µg/mL)	Replicate	TFT Colonies				VC Colonies				Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
		Counts			Mean	Counts			Mean			
0 (solvent)	1	105	76	53	78 ± 21	184	166	137	162 ± 19	96	--	--
0 (solvent)	2	87	78	+	83 ± 4	207	201	140	183 ± 30	90	--	--
Mean Solvent Mutant Frequency= 93												
50	A	132	123	101	119 ± 13	198	146	166	170 ± 21	140	46	67
50	B	92	92	117	100 ± 12	163	172	127	154 ± 19	130	37	56
75	A	133	133	104	123 ± 14	165	171	140	159 ± 13	155	62	41
75	B	77	55	81	71 ± 11	173	159	162	165 ± 6	86	-7	43
100	A	120	129	120	123 ± 4	130	139	117	129 ± 9	191	98	25
100	B				+				+			
125	A	139	127	130	132 ± 5	132	129	149	137 ± 9	193	100	18
125	B	144	71	104	106 ± 30	178	96	145	140 ± 34	152	59	17
150	A	123	92	129	115 ± 16	126	111	145	127 ± 14	180	87	13
150	B	47	109	127	94 ± 34	127	114	125	122 ± 6	155	61	13
Positive Control - Methyl Methanesulfonate (µg/mL)												
10	--	111	235	232	193 ± 58	123	132	129	128 ± 4	301	208	51
20	--	228	190	195	204 ± 17	77	34	39	50 ± 19	817	724	13

Solvent = DMSO

+ = Culture lost

[a] Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[b] Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] % total growth = (% suspension growth x % cloning growth) / 100

Cloning Data for L5178Y/TK$^{+/-}$ Mouse Lymphoma Cells
Treated with TMP
in the Presence of Exogenous Metabolic Activation
Initial Assay (4-hour exposure)

Dose Level (µg/mL)	Replicate	TFT Colonies				VC Colonies				Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
		Counts			Mean	Counts			Mean			
0 (solvent)	1	101	52	103	85 ± 24	195	173	179	182 ± 9	94	--	--
0 (solvent)	2	68	83	111	87 ± 18	166	169	189	175 ± 10	100	--	--
Mean Solvent Mutant Frequency= 97												
10	A	99	97	106	101 ± 4	202	166	201	190 ± 17	106	9	102
10	B	74	59	100	78 ± 17	205	144	190	180 ± 26	86	-10	96
30	A	103	86	114	101 ± 12	159	160	144	154 ± 7	131	34	26
30	B	100	99	90	96 ± 4	175	185	173	178 ± 5	108	12	37
40	A	146	120	103	123 ± 18	173	148	163	161 ± 10	152	56	27
40	B	77	129	132	113 ± 25	192	176	198	189 ± 9	119	23	28
50	A	130	129	120	126 ± 4	190	170	155	172 ± 14	147	50	22
50	B	118	142	109	123 ± 14	177	165	176	173 ± 5	142	46	22
100	A	107	123	99	110 ± 10	175	185	140	167 ± 19	132	35	12
100	B	81	106	55	81 ± 21	171	185	143	166 ± 17	97	0	12
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)												
5	--	142	247	238	209 ± 48	163	104	120	129 ± 25	324	227	49
7.5	--	214	218	245	226 ± 14	61	46	63	57 ± 8	796	700	7

Solvent = DMSO

[a] Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[b] Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] % total growth = (% suspension growth x % cloning growth) / 100

Cloning Data for L5178Y/TK$^{+/-}$ Mouse Lymphoma Cells
Treated with TMP
in the Absence of Exogenous Metabolic Activation
Extended Treatment Assay (24-hour exposure)

Dose Level (µg/mL)	Replicate	TFT Colonies				VC Colonies				Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
		Counts			Mean	Counts			Mean			
0 (solvent)	1	51	39	38	43 ± 6	164	150	140	151 ± 10	56	--	--
0 (solvent)	2	32	31	30	31 ± 1	116	140	140	132 ± 11	47	--	--
Mean Solvent Mutant Frequency= 52												
50	A	42	70	20	44 ± 20	112	119	98	110 ± 9	80	29	61
50	B	28	38	48	38 ± 8	110	100	133	144 ± 14	66	15	64
75	A	89	83	33	68 ± 25	127	122	127	125 ± 2	109	57	70
75	B	73	42	45	53 ± 14	130	137	125	131 ± 5	82	30	67
100	A	86	87	48	74 ± 18	130	138	125	131 ± 5	112	61	63
100	B	84	94	116	98 ± 13	119	122	112	118 ± 4	67	115	61
125	A	44	54	19	39 ± 15	122	140	153	138 ± 13	56	5	58
125	B	27	27	54	36 ± 13	111	110	98	106 ± 6	68	16	48
150	A	72	39	30	47 ± 18	113	139	122	125 ± 11	75	24	28
150	B	40	39	58	46 ± 9	133	123	104	120 ± 12	76	24	31
Positive Control - Methyl Methanesulfonate (µg/mL)												
2.5	--	68	122	150	133 ± 34	130	111	119	120 ± 8	189	137	95
5	--	143	126	76	115 ± 28	80	94	51	75 ± 18	307	255	49

Solvent = DMSO
[a] Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200
[b] Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls
[c] % total growth = (% suspension growth x % cloning growth) / 100

Overall conclusions: All criteria for a valid study were met as described in the protocol. The results of the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay indicate that, under the conditions of this study, the mutagenicity of TMP was concluded to be equivocal without activation with 4- and 24-hour exposures and negative with S9 activation with a
4-hour exposure.

Reference: San, R.H.C. and Clarke, J.J (2002). Unpublished report no AA52LV.704.BTL entitled "In vitro mammalian cell gene mutation test (L5178Y/TK$^{+/-}$ mouse lymphoma assay)" dated November 26, 2002 for General Electric Company, Pittsfield, MA, USA; from BioReliance Corp., Rockville, MD, USA.

Reliability: (Klimisch Code 1) Valid without restrictions.

16.0 REPEATED DOSE TOXICITY

No studies were found

17.0 REPRODUCTIVE TOXICITY

No studies were found.

18.0 DEVELOPMENTAL TOXICITY/TERATOGENICITY

No studies were found.